Lasers in Surgery and Medicine 25:178–186 (1999)

Comparison of Flash Lamp Pulsed-Dye Laser (585 nm) and Conventional Surgery in the Delay of Random Dorsal Rat Flaps

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Background and Objective: **Delay is a basic surgical technique used by flap surgeons to improve the blood supply to the distal parts of a random skin flap. The aim of this study was to determine whether a scarless delay can be done by the use of the flash lamp pulsed-dye laser operating at a wavelength of 585 nm.**
Study Design/Materials and Methods: **The pilot study showed that 6 J/cm^2 had a selective photothermolysis effect and therefore was chosen for testing the delay procedure on 15 rats. The percentage of flap necrosis of this group was compared to the results of 15 rats that underwent delay by surgery and 15 rats that were not treated prior to flap surgery (control group).**
Results: **Laser delay of McFarlane flaps resulted in an average of 15.5% smaller necrotic area compared to the control group (52.7% ± 14.4% and 68.2% ± 9.6%, respectively, $P < 0.01$) and was as effective as surgical delay (53.3% ± 13.6%).**
Conclusions: **The results indicate that the flash lamp pulsed-dye laser operating at 585 nm is effective for delaying cutaneous flaps in the rat model. Lasers Surg. Med. 25:178–186, 1999.**

Key words: animal study; skin flap

Delay is a basic surgical technique used by flap surgeons to improve the blood supply to the distal parts of a random skin flap [1]. It is traditionally done two weeks prior to flap dissection by partial incision of the planned flap borders. This procedure is followed by partial flap ischemia that enhances, by an uncertain mechanism, the blood supply to the distant parts of the flap [1]. Delay is used less frequently today because it requires a preoperative commitment to a distinct flap outline, has to be performed in a sterile environment, and is associated with the possible complications of a surgical procedure—pain, bleeding, infection, and scarring. If during operation the surgeon, based on the actual size of the defect, decides not to use the flap then the patient is left with a superfluous scar.

Our objective was to find a scarless technique for delay that would also be pain-free, bloodless, infection-free, and would not necessi-

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Accepted 3 December 1998

tate a sterile environment. The pulsed-dye laser, operating at a 585 nm wavelength, has selective thermocoagulative effects on the subpapillary and subdermal blood vessels which supply random skin flaps [2–5], and therefore is a potentially excellent option for delaying random skin flaps.

Odland and associates studied a rat McFarlane flap model and found that the delay by a continuous wave argon pumped-dye laser operating at 577 nm was as effective as the delay by surgery [6]. Using this laser, one may obtain a pulsed or continuous (CW) light of various wavelengths covering the visible spectral range. Continuous dye laser often exceeds the estimated 1 millisecond thermal relaxation time of cutaneous microvessels [7] and therefore has a reduced selective photothermolysis effect on the skin vessels compared with a pulsed dye device [8]. Not surprisingly, Odland and associates found superficial thermal injury to the skin, full thickness adhesions in the plane between the panniculus carnosus and deep muscle fascia, destruction of dermal collagen, vesicle formation, and partial separation of the dermal-epidermal junction [6]. These findings were reported to represent nonselective skin injury associated with dermal fibrosis and scarring [8–11], events that obviously limit the use of laser for this purpose in the field of Plastic Surgery. Another disadvantage of the device employed by Odland and his associates was their use of the 577 nm wavelength [6]. It was found that dye laser operating at 577 nm has an effect limited to the human midreticular dermis without causing subdermal plexus coagulation [10–12]. A change in the wavelength from 577 nm to 585 nm allowed the laser light to penetrate from the midreticular dermis into the subcutaneous fat and coagulate the subdermal plexus [12].

We assumed that a pulsed-dye laser operating at 585 nm would be more suitable for examining the delay by laser. Our aim was to initially find the flash lamp (585 nm) pulsed-dye laser energy that would have an exclusive effect on the rat subpapillary and subdermal blood plexuses, without any concomitant destruction of the surrounding tissue. We intended to find if such an energy level could be used successfully to delay a random rat skin flap.

MATERIALS AND METHODS

Laser Equipment

A flash lamp pumped pulsed-dye laser, operating at 585 nm, 1 pulse per second and a pulse duration of 450 seconds (Photogenica 5, Laser Industries™, Tel-Aviv, Israel) was used. The laser beam was transmitted through an optical fiber and then magnified to a 3-mm spot diameter on the skin.

Animals

Male Wistar rats weighing 350 g were studied. They were kept in separate boxes and fed commercial rat food and water ad libitum. The study was approved by the Animal Research Committee at the Tel-Aviv Sourasky Medical Center, Tel-Aviv.

Dye Laser Calibration

To determine if the flash lamp pumped pulsed-dye laser energy at 585 nm has an exclusive effect on the rat subpapillary and subdermal blood plexuses without surrounding tissue destruction, shaved dorsal skin areas of 0.6 × 0.9 cm of four rats were treated with the application of six pulses of energies: 2 joules/cm^2/pulse, 4 joules/ cm^2/pulse, 6 joules/ cm^2/pulse, or 8 joules/ cm^2/pulse. Each laser energy was applied at two separate areas. The animals were killed one hour, three days, seven days, and two weeks following the laser treatment.

Biopsies were taken from the eight lased areas and two additional unlased control areas in each animal. Each sample was cut into four specimens (32 specimens for each laser energy and 32 control specimens yielding a total of 160 specimens). These specimens were evaluated by hematoxylin-eosin-stained slide microscopy. The most severe microscopic changes were recorded.

The macroscopic appearances of the rat skin at one hour, three days, seven days, and 14 days following treatment were registered. The most severe changes were recorded and photographed.

Flap Delay

In an early stage we recognized that 6 J/ cm^2 of flash lamp (585 nm) pulsed-dye laser energy had an exclusive effect on the rat subpapillary and subdermal blood plexuses. This will be fully discussed in the Results section. We intended to find if this energy level could be used successfully to delay a random rat skin flap.

Two weeks before flap surgery, all 45 animals were anesthetized (intraperitoneal ketamine 125 mg/kg and xylasine 7 mg/kg). The dorsal hair was shaved and then completely removed by commercial depilatory cream application. All animals had a dorsal cutaneous, cranially based, Mc-

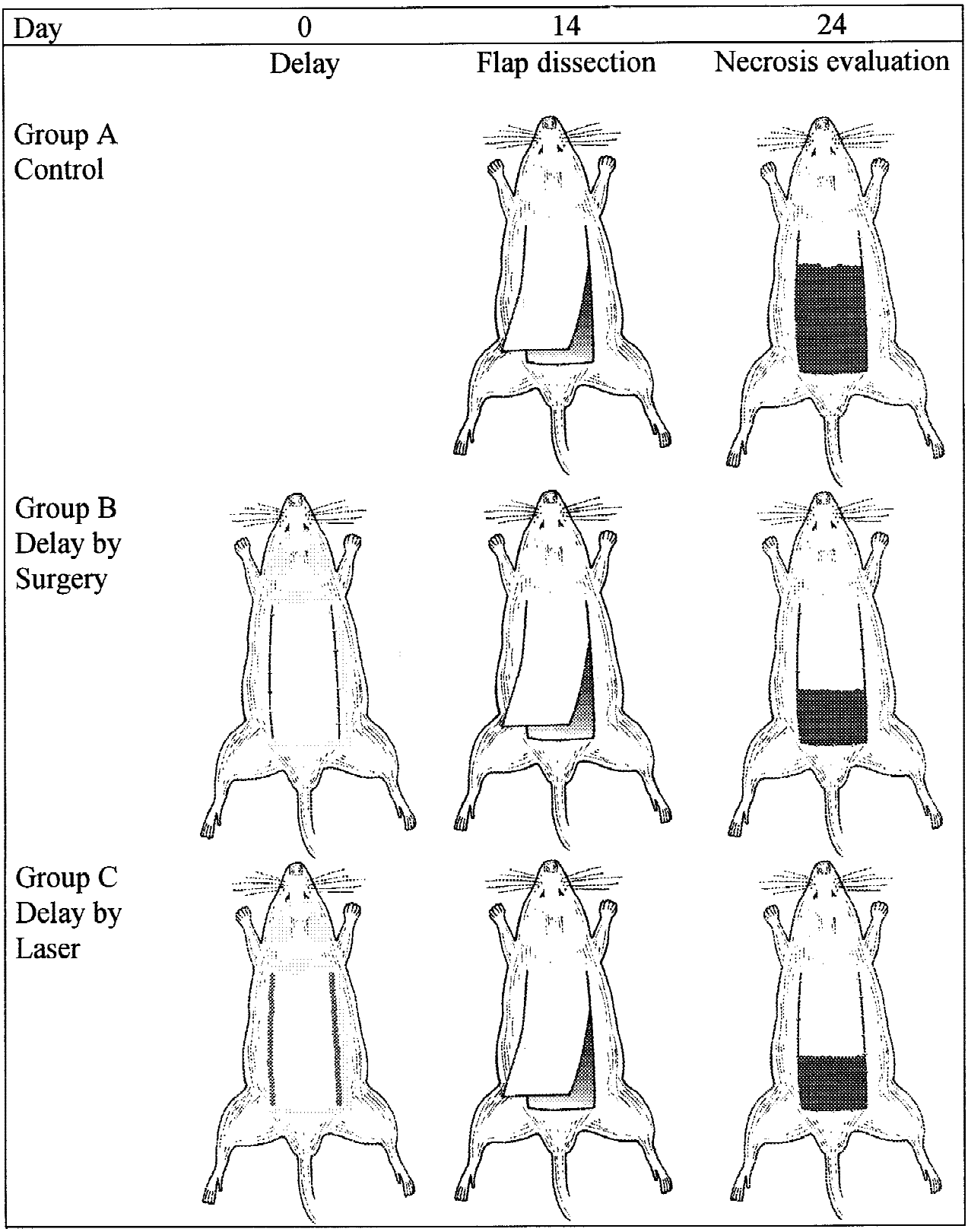

Fig. 1. Study design. Dashed lines indicate skin incision. Bold, gray lines indicate laser treatment.

TABLE 1. Microscopic Changes Associated With Various Dye Laser Energy Densities 1 Hour, 3 Days, 7 Days, and 14 Days Following Laser Treatment

Dye Laser Energy Density J/cm^2	Time From the Laser Treatment			
	1 hour	3 days	7 days	14 days
2	No effect	No effect	No effect	No effect
4	No effect	Few subpapillary vessels with thrombi	No effect	No effect
6	Subpapillary thrombi (Fig. 2)	Subpapillary and subdermal vessels with thrombi, some with polymorphonuclear leukocytes	Subpapillary and subdermal vessels with thrombi, some with fibroid degeneration of vessel wall	Subpapillary and subdermal thrombi, fibroid degeneration of vessel wall
8	Subpapillary and subdermal vessels with thrombi, focal dermal-epidermal separation, widespread degeneration of dermal collagen	Subpapillary and subdermal vessels with thrombi, ectatic blood vessels, fibroid necrosis of blood vessel wall, separation of epidermal corneal layer, widespread degeneration of dermal collagen, polymorphonuclear leukocytes	Subpapillary and subdermal vessels with thrombi, ectatic blood vessels, fibroid necrosis of blood vessel wall, separation of epidermal corneal layer, focal absence of the epidermis	Local destruction of skin architecture, partial replacement of normal architecture by fibroblasts, absence of the epidermis

Farlane flap marked with permanent pen at six points over their back [13,14]. Fifteen rats (group A) underwent anesthesia, shaving, and application of the depilatory cream (control group in Fig. 1). An additional 15 rats (group B) underwent incision of the longitudinal flap borders, including the panniculus carnosus without flap undermining (delay by surgery group in Fig. 1). The incisions were sutured with 4/0 nylon stitches. The remaining 15 rats (group C) were treated over the longitudinal borders of the marked McFarlane flaps with 3 pulses/cm of 6 joules/cm^2/pulse dye laser energy ("delay by laser" in Fig. 1). All animals were kept in separate boxes and fed commercial rat food and water ad libitum.

Flap Elevation

Two weeks following the primary treatment, all animals underwent McFarlane flap elevation according to the pen marks. The flap was raised with the panniculus carnosus and sutured back with 4/0 nylon sutures. Two rats of group B expired following flap dissection.

The total size and the necrotic part of the flap were marked on cellulose paper 10 days later. The cellulose paper areas were weighted and the percentage of flap necrosis was calculated.

Statistical Analysis

Multiple comparisons between the percentage of necrosis values of groups A, B, and C were performed using one-way analysis of variance (ANOVA) with Bonferroni's P value. A Bonferroni P value of less than 0.05 was considered significant.

RESULTS

The microscopic appearances of the skin following various energies of pulsed-dye laser application are presented in Table 1 and Figures 2 and 3. This shows that selective photothermolysis was associated with 6 J/cm^2 dye laser energy application. Lower energy densities had an insignificant effect on blood vessels, whereas a higher density was associated with nonselective destruction of the surrounding tissue. The blood vessels affected by the 6 J/cm^2 pulses were located in the subpapillary (Fig. 2) and subdermal (Fig. 3) plexuses.

The macroscopic changes associated with various pulsed-dye laser energies are presented in Table 2 and Figure 4. The results indicate that energy density of 8 joules/cm^2 was associated with burns and skin ulceration as demonstrated microscopically.

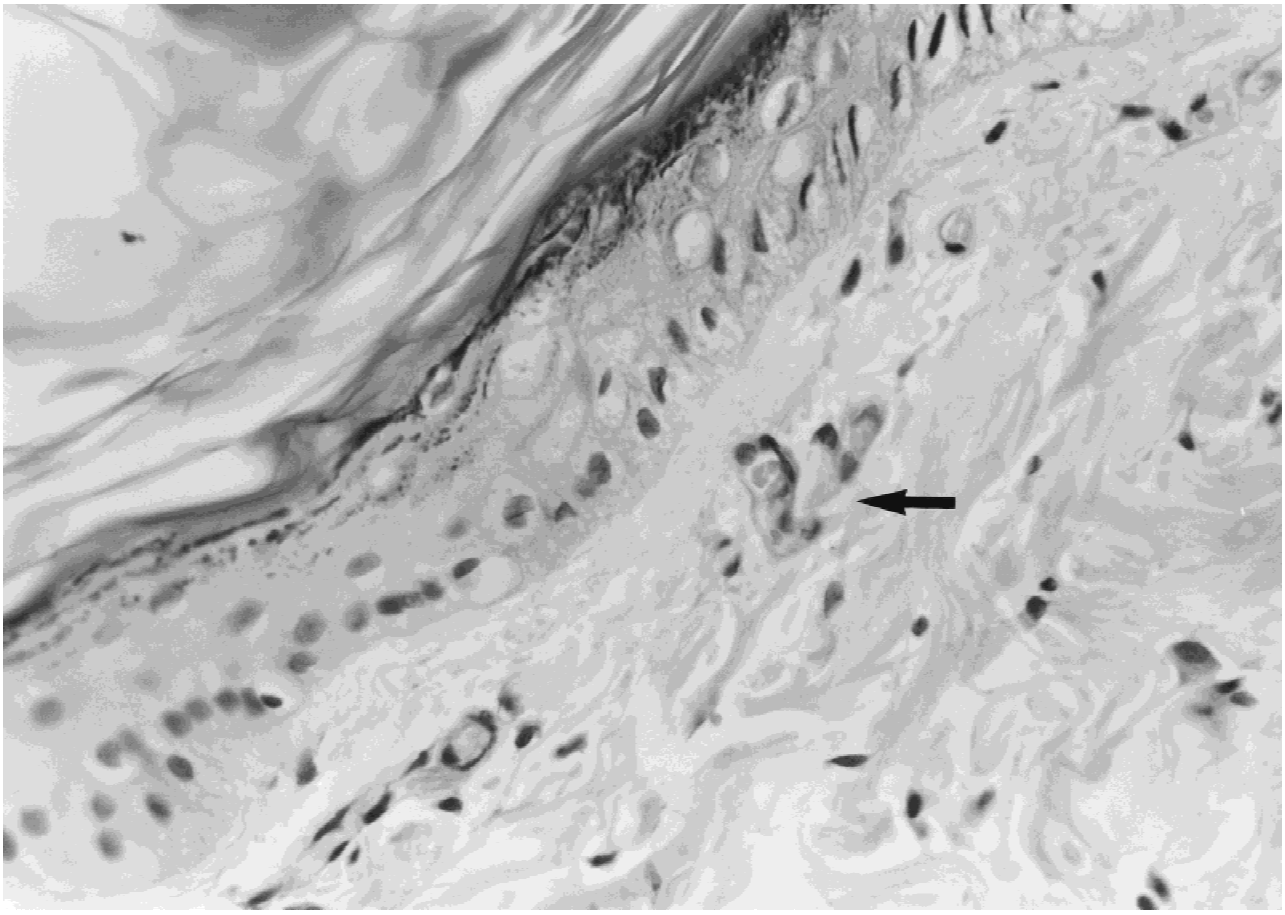

Fig. 2. Dilated subpapillary capillaries (arrow) containing agglutinated and intact red blood cell thrombi immediately following exposure to 6 J/cm^2 dye laser (hematoxylin-eosin, ×400).

The percentages of flap necrosis of groups A, B, and C are presented in Table 3. This shows that dye laser delay of the McFarlane flaps resulted in a 15.5% smaller area of necrosis compared to the control group. This difference was highly significant statistically ($P < 0.001$). Delay by surgery was associated with a 15% smaller flap necrosis. This difference was statistically significant as well ($P < 0.05$). The 0.6% difference between the delay by laser and the delay by surgery groups was not statistically significant.

DISCUSSION

The present study confirms that pulsed-dye laser, operating at 585 nm, is an effective tool for delaying a rat McFarlane flap. We believe that this method can also be used in humans. It should be mentioned that some authors believe that the "loose skin" rat has a significantly different blood supply to the skin, compared with the human system [5,15], others think that the rat dorsal cutaneous flap relies on musculocutaneous perforators [14,16] and has a subpapillary and a subdermal vascular plexuses [12] similar to those in a human being.

Another difference between human random skin flaps and the McFarlane flap, used in this study is the presence of a thin muscle layer (panniculus carnosus) in the rat flap [14]. As it relates to the blood supply to the skin, this layer is compatible with the panniculus adiposus of the human skin [13]. McFarlane et al. thought that a dorsal flap must include this layer in order to contain the subdermal plexus [13]. We found that the inclusion of this layer in the delay incision had no significant effect on flap survival. This is supported by the almost identical percentage of flap survival of groups B and C. This similarity was not dependent on whether the panniculus carnosus was included in the delay procedure (group B) or not (group C).

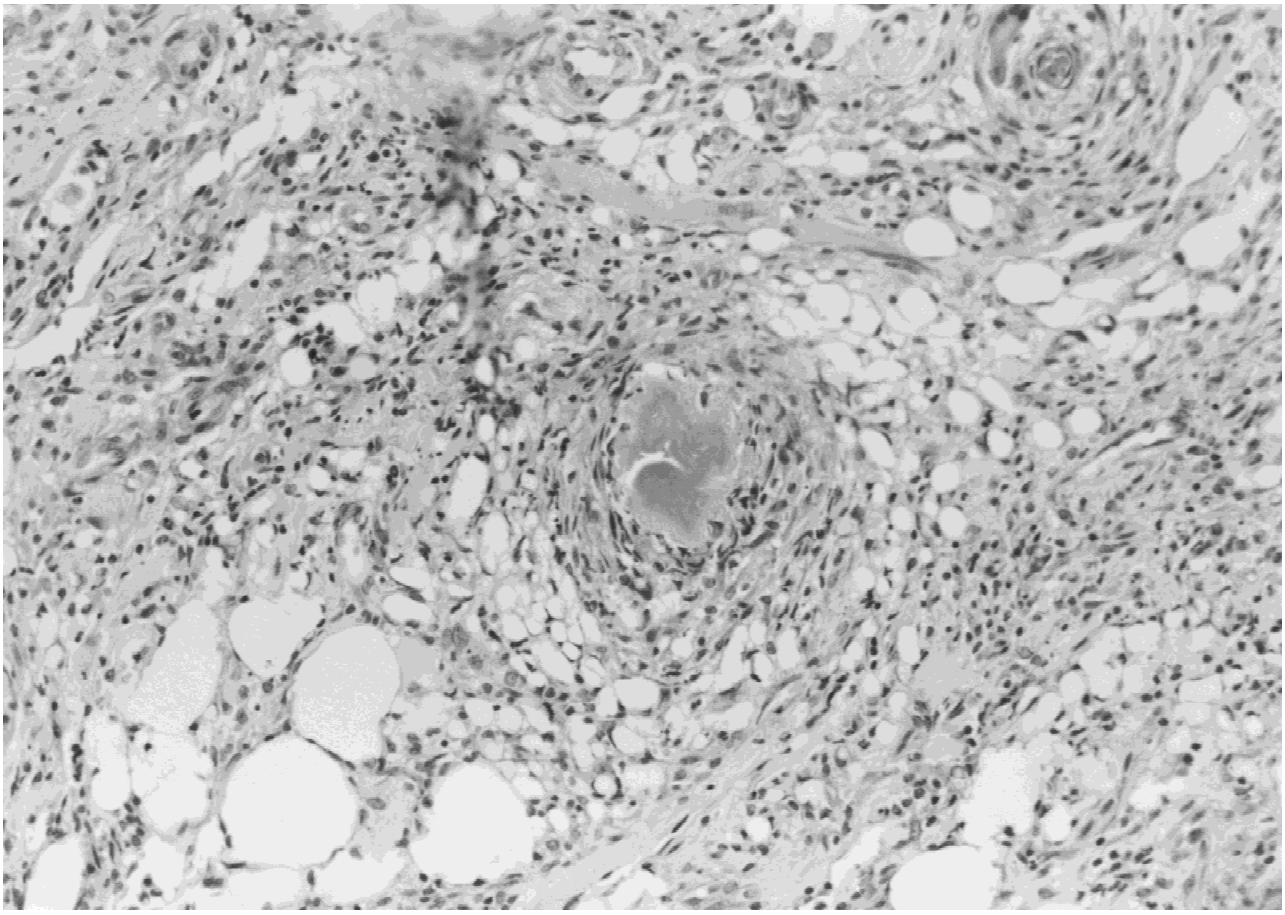

Fig. 3. Deep dermis ectatic blood vessel with thrombus seven days following exposure to 6 J/cm^2 dye laser. Normal appearance of adjacent tissue (hematoxylin-eosin, ×200).

TABLE 2 Macroscopic Changes Associated With Various Dye Laser Energy Densities 1 Hour, 3 Days, 7 Days, and 14 Days Following Laser Treatment

Dye Laser Energy Densities J/cm^2	Time From the Laser Treatment			
	1 hour	3 days	7 days	14 days
2	No effect	No effect	No effect	No effect
4	No effect	No effect	No effect	No effect
6	Blue-gray discoloration	Fine brown-gray discoloration	Fine brown discoloration	No effect
8	Prominent gray discoloration	Crust	Crust	Superficial skin ulceration

Pulsed-dye laser application to delay normal skin is performed differently from the care of vascular lesions of the skin:

Use of relatively low energy: Although there is a direct ratio between dye laser wavelength value and the energy required to affect the blood vessels of the skin [11], vascular coagulation was achieved in this study following a relatively low energy density application (6 J/cm^2). Based on previous microscopy studies that showed vascular specific damage following application of a low (577 nm wavelength) dye laser energy density (up to 3 J/cm^2) [7,8,10,11], and from the relatively low energy used in this study, we concluded that the energy density required for a successful delay procedure is low. This is probably lower than the average value required

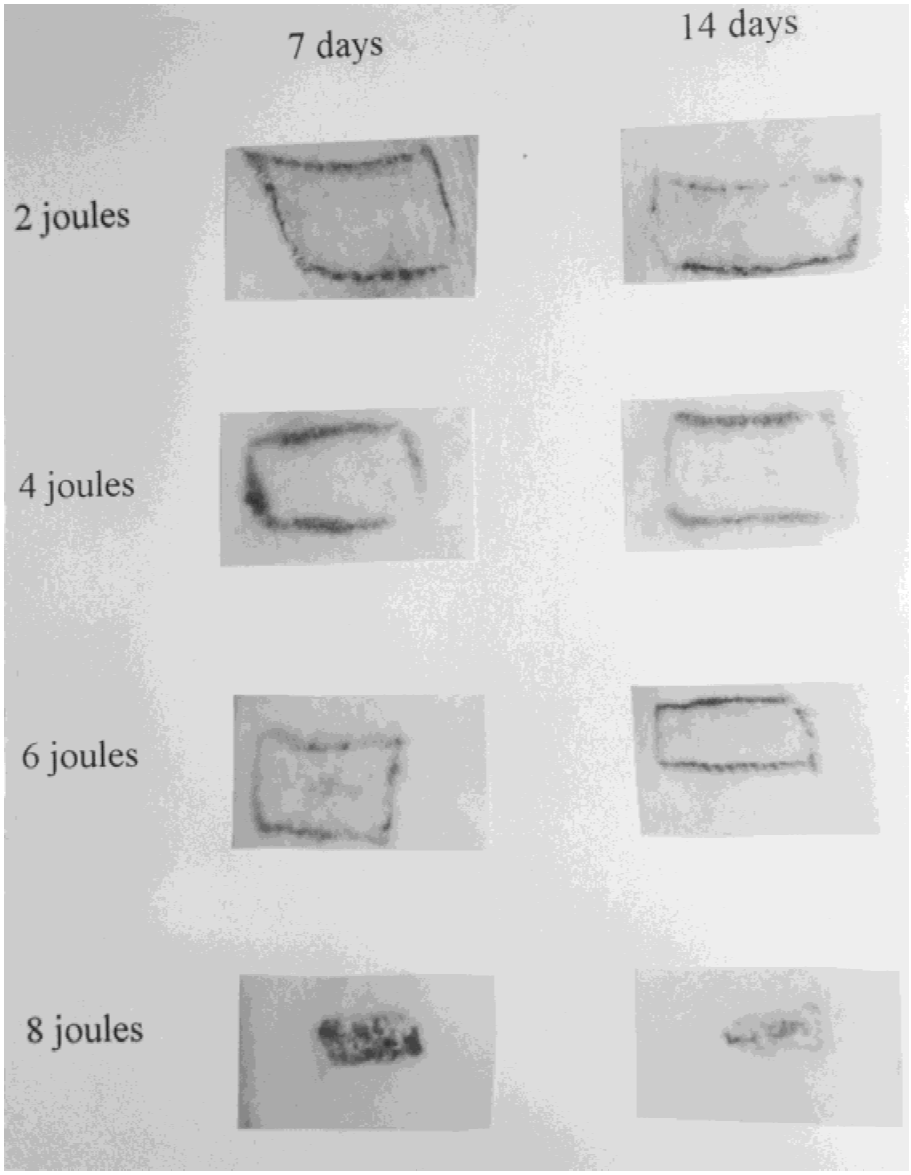

Fig. 4. The effect on the skin observed one week and two weeks following pulsed dye application. Note that the brown discoloration observed one week following 6 J/cm^2 dye laser energy application was not apparent at the following week. Application of 8 J/cm^2 caused burns and skin ulceration.

TABLE 3. Percent of Flap Necrosis Among the Delay by Surgery, Delay by Laser, and Control Groups

Group	Number of Animals	Mean % of Flap Necrosis	SD[a] of the Mean % of Flap Necrosis
Delay by surgery	13	53.3	13.6
Delay by laser	15	52.7	14.4
No delay (control)	15	68.2	9.6

[a]SD: Standard deviation.

for the treatment of the dilated, aberrant vessels present, for instance, in a port wine stain [4].

Use of a marker and a large spot size: One of the advantages of the delay by pulsed-dye laser is the absence of skin surface signs. Ironically, this advantage becomes a problem when flap elevation is performed. An easy solution is the use of a high quality permanent pen to mark key points on the flap border (Fig. 5). We also recommend using a relatively large spot size (at least 3 mm in diam-

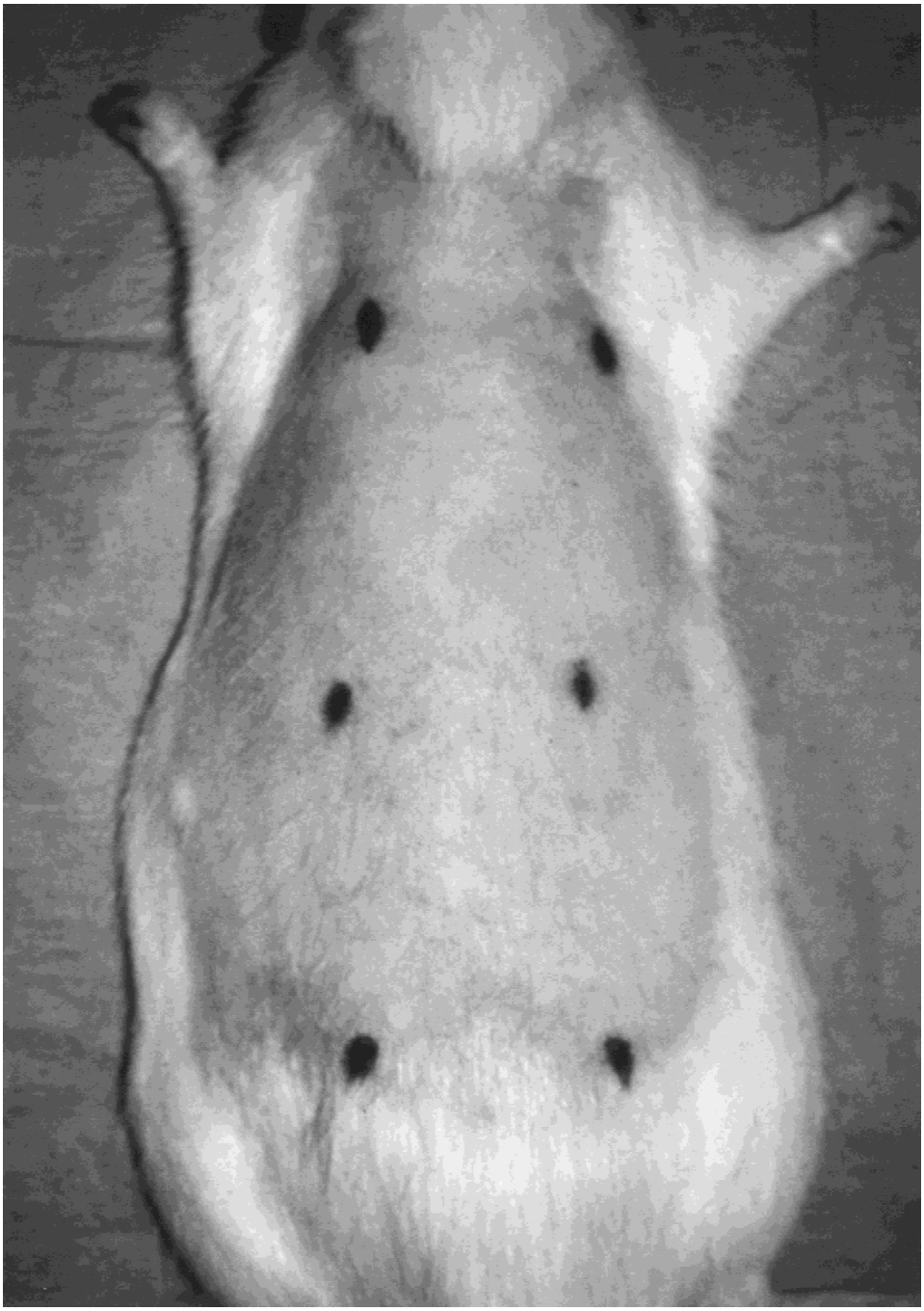

Fig. 5. Intact skin is observed two weeks following pulsed dye laser delay of the McFarlane flap.

eter) during the delay procedure. This decreases the chance of missing the "invisible" treated area.

The laser method presented has several potential advantages over current delay techniques. These include low cost, lack of bleeding, and no infection. It also does not obligate a sterile environment and so can be performed as an office procedure without anesthesia. The possible avoidance of scarring can afford the delay of several different flaps for future reconstruction of a single defect. For example, if excision of a nasal tip basal cell carcinoma is anticipated, a preceding delay procedure can include laser delay of both an extended bi-lobed nasal flap and a very long nasolabial flap. Following tumor excision, the surgeon can select the flap that fits the nasal defect best.

The results of this animal study are promis-

ing, but further studies in humans are required to confirm the proposed advantages suggested. Those studies should specifically be aimed at determining the optimal dose and pulse width necessary for delaying random skin flaps without causing nonselective destruction and scarring.

ACKNOWLEDGMENT

We thank Mrs. Ester Eshkol for her assistance in the preparation of this manuscript.

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